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Original Article

Safety and early treatment effects of the CXCR2 antagonist SB-656933 in patients with cystic fibrosis

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Abstract

Background: It is hypothesized that a CXCR2 receptor antagonist would inhibit the recruitment and activation of neutrophils and other inflammatory cells into the lung in subjects with cystic fibrosis. The objective of this study was to evaluate the safety, tolerability and pharmacodynamics of SB-656933, an oral CXCR2 antagonist.
Methods: 146 adult CF patients were randomized to receive either placebo or SB-656933 20 mg or 50 mg once daily for 28 days. The primary endpoint was safety; secondary endpoints included pharmacokinetics, blood and sputum biomarkers, sputum microbiology, pulmonary function and respiratory symptoms.
Results: SB-656933 was generally well tolerated. The most frequent adverse event was headache. Five subjects were withdrawn due to adverse events. In subjects receiving SB-656933 50 mg, sputum neutrophils and elastase were reduced compared to baseline (probability of a true reduction, 0.889 and 0.882 respectively), and free DNA reduced compared to placebo (probability of a true reduction, 0.967), while blood levels of fibrinogen, CRP and CXCL8 were increased. There were no changes in lung function or respiratory symptoms. Average plasma concentrations of SB-656933 were lower than predicted based on previous studies, only breaching IC_{50} for ~4 h at the 50 mg dose.
Conclusions: SB-656933 was well-tolerated in adult patients with cystic fibrosis. Patients receiving a daily dose of 50 mg showed trends for improvement in sputum inflammatory biomarkers despite potential blunting of effects by lower than expected plasma concentrations. Although the increase in systemic inflammatory markers requires further evaluation, CXCR2 antagonism may be a useful approach for modulating airway inflammation in patients with cystic fibrosis. Clinical trial registered with www.clinicaltrials.gov (NCT00903201).

Keywords: CXCR2; Cystic fibrosis; Chemokine; Clinical trial; Human; Inflammation

1. Introduction

Cystic fibrosis (CF) is the most common lethal autosomal recessive disease among Caucasians. CF lung disease is characterized by airway obstruction, infection, neutrophilic inflammation and progressive bronchiectasis. Median survival is approximately 37 years with over 90% of patients dying from complications of pulmonary disease. It is postulated that the recruitment and activation of inflammatory cells in the airways contribute significantly to the pulmonary pathophysiology of CF and that the degree of inflammation is excessive [1]. This was the rationale

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behind studies of systemic steroids [2], ibuprofen [3] and azithromycin [4]. To date, no anti-inflammatory therapy has been shown to convincingly reduce markers of airway inflammation in CF, however, several approaches are being explored [5].

Small studies in CF demonstrated an inverse relationship between lung function and sputum levels of CXCL8 (IL-8), total cell counts, neutrophil counts, neutrophil elastase, and myeloperoxidase [6–8]. CXCL8 was the first identified member of a growing family of pro-inflammatory chemokines that attract and activate immune and inflammatory cells, particularly neutrophils. The CXC chemokines function as agonists at G-protein-coupled transmembrane cell surface receptors on target cells. The CXC receptors (CXCR1 and CXCR2) are located on neutrophils, subsets of T-cells, macrophages, dendritic cells, and mast cells. Their agonists contain the glutamic acid–leucine–arginine (ELR+) sequence before the first cysteine residue. CXCR1 is selectively activated by CXCL6 and CXCL8, while the CXCR2 receptor is stimulated potently by CXCL1-3, and CXCL5-8 [9].

Based upon pre-clinical evidence, it has been proposed that a CXCR2 receptor antagonist would specifically inhibit the recruitment and activation of neutrophils and other inflammatory cells into the lung in subjects with CF. SB-656933 is a selective CXCR2 antagonist in development as a novel, once-daily oral anti-inflammatory agent for the maintenance treatment of CF [10,11]. It is hypothesized that selective antagonism of CXCR2, by inhibiting neutrophil recruitment but not microbial killing (thought to be mediated by CXCR1) [12], would potentially restore the balance between host defense and tissue damage mediated by neutrophil products. The compound represents a novel class of agents compared with those previously approved for CF and by inhibiting airway inflammation over time would be expected to stabilize lung function.

2. Methods

2.1. Study design

This study (GSK protocol CF2110399, NCT00903201) was a randomized, double blind, parallel group, placebo-controlled trial with 3 treatment arms (Fig. 1). Subjects were recruited from 31 sites in the US, France, Germany, and Israel. All subjects attended a screening visit (Visit 1) at which time their eligibility for study inclusion was assessed. Baseline assessments included spirometry, induced sputum and sampling for blood biomarkers. Eligible subjects were randomized to receive either placebo or active treatment for 28 days. On Day 14 (Visit 3), assessments included spirometry and sampling of blood for biomarkers and pharmacokinetics. On Day 28 (Visit 5), end of treatment assessments included induced sputum and sampling of blood for biomarkers and pharmacokinetics. Patients completed a daily symptom diary throughout the treatment period. Subjects also returned for a follow-up visit (Visit 6) approximately 7 to 14 days after last dose of study medication.

The study protocol, any amendments, informed consent, and other information that required pre-approval were reviewed and approved by a national, regional, or investigational center ethics committee. The study was conducted in accordance with good clinical practice and all regulatory requirements, including, where applicable, those originating from the Declaration of Helsinki. All subjects provided written informed consent before treatment.

2.2. Endpoints

The primary endpoint for this study was the safety of SB-656933 in subjects with cystic fibrosis, including adverse events, vital signs and clinical laboratory assessments, electrocardiographic (ECG) parameters, and CF exacerbations (including withdrawals, time to exacerbations and/or new antibiotic prescription).

Secondary endpoints included: 1) qualitative sputum microbiology for *Pseudomonas aeruginosa* and *Staphylococcus aureus*; 2) induced sputum neutrophil number (cells/mL) and percentage; 3) sputum inflammatory biomarkers (neutrophil elastase (NE), myeloperoxidase (MPO), free DNA and PGP peptides); 4) serum and plasma markers of inflammation (fibrinogen, CRP, CC-16, MMP8, MMP9, SP-D, CXCL8); 5) forced expiratory volume in one second (FEV_1) and forced vital capacity (FVC); and 6) plasma SB-656933 concentrations and pharmacokinetic parameters including area under the plasma drug concentration *versus* time curve (AUC_{0-4}, AUC_{0-t}), maximum observed plasma drug concentration (C_{max}) and time to maximum observed plasma drug concentration (T_{max}). The Daily Respiratory Symptom Diary for Cystic Fibrosis (Self Reported Version) was included as an exploratory endpoint [13].

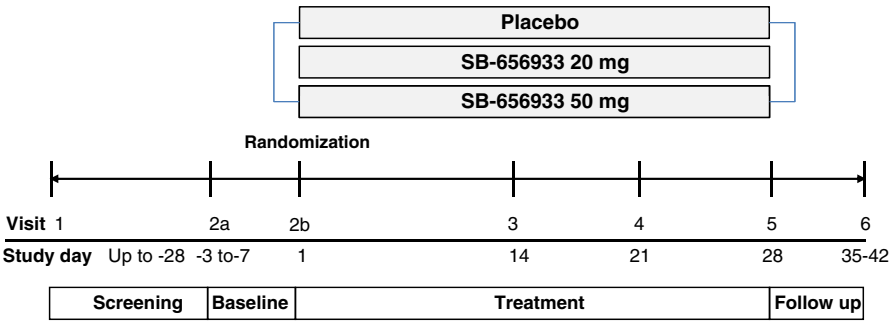

Fig. 1. Study schematic.

2.3. Study population

Males or females ≥18 years of age with a diagnosis of CF were eligible to participate. Subjects were not permitted to be on inhaled antibiotics during the study commencing from one week prior to dosing until the final PK draw, in order to minimize variability. Maintenance therapy with inhaled hypertonic saline, inhaled DNase or inhaled corticosteroids was permitted provided there were no changes to therapy during the study. A detailed description of inclusion and exclusion criteria is available in the online supplement.

2.4. Biomarkers

Detailed methods for sputum processing and biomarker analysis can be found in the online supplement.

2.5. Respiratory symptoms

The "Daily Respiratory Diary Card for Cystic Fibrosis — Self Reported (Ages 12+ years)", also referred to as CFRSD (Copyright 2007 University of Washington) [13] was completed by all subjects. A detailed description is available in the online supplement.

2.6. Statistical methods

The sample size was primarily based on feasibility since it was not viable to power the study on the primary endpoints of safety and tolerability or with respect to any of the sputum or blood biomarkers. Given the small number of subjects enrolled in this study, these data presented in summary fashion, as there was insufficient power to perform a formal statistical analysis of safety.

A pre-specified log transformation was applied to all biomarker data to improve compatibility with the normal distribution assumption. The biomarker data are presented as geometric means. An analysis of neutrophil cell counts and ratio to baseline was performed for $\log_e$-transformed data using a fixed effects repeated measures analysis, including treatment group and visit as fixed effects. Pre-treatment sputum neutrophil numbers were fitted as a response (all baseline data were assigned to one treatment group called baseline, regardless of the treatment the subject went on to receive, and these values were also fitted as a response). Estimates of the treatment differences (SB-656933 *vs.* placebo) were calculated and presented with two-sided 90% confidence intervals (using the pooled estimate of variance). Bayesian probabilities were produced to provide the probability of SB-656933 demonstrating a true treatment effect greater than placebo. Non-informative priors were used reflecting that no prior information on the treatment effect was made in the analyses. With non-informative priors, these results approximate to 1 minus a 1-sided p-value. The same method of analysis was applied to the remaining sputum biomarkers, as well as to serum and plasma biomarkers. Where appropriate, BQL values were imputed with 1/2 LLQ limit value and AQLs were imputed with the AQL limit value.

An exploratory TOBIT analysis was performed on biomarkers CRP, CXCL-8, free DNA, MPO, NE and SP-D, as these data incurred values below the quantification limit (BQL) and/or above the quantification limit (AQL). TOBIT analysis is an optimization procedure that obtains parameter estimates from a model that describes the distribution by fitting the model to both the observed and unobserved data; this type of analysis enables more accurate estimation of the mean and variance of a truncated distribution, as in the case with BQLs and AQLs. A table quantifying the number of out-of-range data points is included in the online supplement (Table S1).

Lung function data (change from baseline FEV_1 and FVC) were analyzed in a similar way to that described for sputum neutrophils. However, the statistical analysis for change from baseline lung function measures included the covariate "day" as a fixed effect in the model as well.

Two interim analyses were performed during the conduct of this study. A detailed description of the interim analysis plan can be found in the online supplement.

3. Results

3.1. Safety

A total of 146 subjects were randomized according to a planned 4:3:3 placebo:active arms ratio (Figure S1). Of these, 61 subjects received at least one dose of placebo, 44 subjects received at least one dose of SB-656933 20 mg and 41subjects received at least one dose of SB-656933 50 mg. Demographic information is included in Table 1.

There were a similar number of reported adverse events across all treatment groups (Table 2). The most frequent AE reported across all groups was headache. There were trends for increased incidence of pyrexia, hemoptysis, and nasopharyngitis in the subjects receiving SB-656933, but these were not considered drug-related. The most frequent drug-related AE reported across all groups was also headache.

Five subjects were withdrawn from the study due to AEs that were considered study drug related, including one on placebo (CF exacerbation) and 4 receiving SB-656933 20 mg: throat tightness (one patient); testicular pressure (one patient); abdominal pain,

Table 1
Patient baseline demographic and clinical characteristics.

	Placebo (n=61)	SB-656933 20 mg (n=44)	SB-656933 50 mg (n=41)
Age, years (range)	29.5 (18–54)	32.9 (18–70)	31.3 (18–63)
Male %	64	64	59
FEV_1 % predicted (range)	66.5 (61.9–71.1)	69.8 (64.2–75.3)	65.4 (59.1–71.7)
BMI, kg/m^2 (range)	22.58 (17–41.9)	22.93 (18.5–34.2)	22.88 (17.7–34.6)
Azithromycin, n (%)	35 (57%)	30 (68%)	18 (44%)
Pulmozyme, n (%)	31 (51%)	25 (57%)	29 (71%)
ICS, n (%)	31 (51%)	22 (50%)	20 (49%)
Pseudomonas in sputum, n (%)	37 (61%)	27 (61%)	25 (61%)

Table 2
Adverse events occurring ≥5% in any group.

System organ class (preferred term)	Placebo (n=61)	SB-656933 20 mg (n=44)	SB-656933 50 mg (n=41)
Any event	46 (75%)	32 (73%)	32 (78%)
Respiratory, thoracic and mediastinal disorders			
Cough	12 (20%)	5 (11%)	7 (17%)
Sputum increased	9 (15%)	2 (5%)	2 (5%)
Oropharyngeal pain	4 (7%)	1 (2%)	3 (7%)
Hemoptysis	1 (2%)	4 (9%)	2 (5%)
Nasal congestion	3 (5%)	0	3 (7%)
Pulmonary congestion	0	1 (2%)	2 (5%)
Rhinorrhea	0	1 (2%)	2 (5%)
Nervous system disorders			
Headache	16 (26%)	13 (30%)	7 (17%)
General disorders and administration site conditions			
Chest discomfort	5 (8%)	3 (7%)	5 (12%)
Pyrexia	0	4 (9%)	2 (5%)
Infections and infestations			
Infective pulmonary exacerbation of cystic fibrosis	5 (8%)	4 (9%)	5 (12%)
Nasopharyngitis	3 (5%)	4 (9%)	4 (10%)
Gastrointestinal disorders			
Diarrhea	3 (5%)	3 (7%)	3 (7%)
Constipation	1 (2%)	1 (2%)	2 (5%)
Flatulence	1 (2%)	0	2 (5%)
Investigations			
C-reactive protein increased	1 (2%)	0	2 (5%)
Musculoskeletal and connective tissue disorders			
Musculoskeletal pain	0	0	2 (5%)
Reproductive system and breast disorders			
Scrotal pain	0	0	1 (2%)
Spontaneous penile erection	0	0	1 (2%)
Testicular pain	0	1 (2%)	0

back pain, fatigue, nasal dryness, headache, epistaxis and sinus headache (single patient); mood swings, fatigue, irritability and depression (single patient).

A total of six subjects (two on each treatment regimen) reported serious adverse events. These included: CF exacerbation (two on placebo, one each on SB-656933 20 mg and 50 mg), intestinal obstruction (SB-656933 20 mg) and hemoptysis (SB-656933 50 mg). None of the CF exacerbations were associated with new infiltrates on CXR. All SAEs were resolved and there were no deaths.

There were no clinically meaningful findings following review of clinical laboratories, vital signs or ECGs for any subject in this study. All individual subject blood neutrophil counts remained in the non-neutropenic range throughout this study, with no subject having an absolute blood neutrophil count below the threshold of 1.5×10^9 cells/L. Finally, there was no overall change in bacterial colonization or semi-quantitative density across the treatment groups (data not shown).

3.2. Sputum biomarkers

Treatment with SB-656933 50 mg for 28 days resulted in a reduction of sputum neutrophils by 31% (90% CI −48, −10) compared to baseline and by 23% (90% CI −45, +9, probability=0.889) compared to placebo (Fig. 2). *Ad hoc* analysis suggested that these results were driven by two responders who appeared to be outliers, despite baseline cell counts that were consistent with those of other subjects and no a priori reason to expect they were anomalous. In contrast, sputum neutrophils were unexpectedly increased in patients receiving SB-656933 20 mg compared to baseline and to placebo (+26%, 90% CI −11, +78, probability=0.133) (Fig. 2). There was a slight decrease observed in percentage neutrophils compared to baseline for all three treatment groups; however, there was no treatment difference observed on Day 28 when comparing SB-656933 20 mg or SB-656933 50 mg with placebo (data not shown). A similar pattern of results to those for sputum neutrophil numbers was observed for sputum macrophages and for the total cell count (data not shown).

Levels of sputum neutrophil elastase were decreased by 26% (90% CI −44, −2) compared to baseline and by 23% (90% CI −46, +11, probability=0.882) compared to placebo in subjects receiving SB-656933 50 mg (Fig. 2). There was a trend for a decrease in sputum MPO compared to baseline (−15%; 90% CI −30, +2) and to placebo (−17%; 90% CI −33, +4, probability=0.914) (Fig. 2). The 50 mg dose also resulted in a significant decrease in sputum free DNA when compared to placebo (−25%; 90% CI −43, −3, probability=0.967), though not when compared to baseline (data not shown). This is likely due to the fact that free DNA levels increased over time in

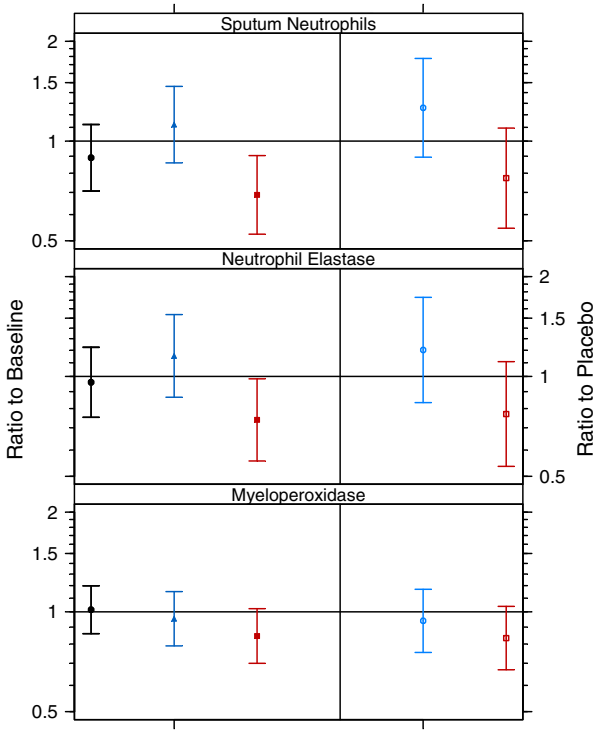

Fig. 2. The effect of SB-656933 on sputum biomarkers at Day 28. Left: adjusted geometric mean±90% CI of the ratio to baseline for neutrophil numbers (top), neutrophil elastase (middle) and myeloperoxidase (bottom). ●—placebo, ♦—SB-656933 20 mg, ■—SB-656933 50 mg. Right: adjusted geometric mean±90% CI for treatment ratio to placebo for SB-656933 20 mg (○) and SB-656933 50 mg (□).

subjects on placebo (+24%; 90% CI: + 5, +48), while they remained stable in subjects receiving the higher dose of SB-656933. There were no differences in free DNA between placebo and SB-656933 20 mg at Day 28 (data not shown).

Sputum PGP on Day 28 was increased compared to baseline for all three treatment groups (Supplement Table 2). However, there was no treatment difference observed on when comparing SB-656933 20 mg or 50 mg with placebo.

3.3. Blood biomarkers

In subjects receiving SB-656933 50 mg, there was an average increase in serum fibrinogen of 14% (90% CI +9, +20) on Day 28 when compared to baseline and an increase of 11% (90% CI+4, +18, probability=0.996) when compared to subjects on placebo (Fig. 3). The pattern of results for CRP was similar but more pronounced than those observed for fibrinogen, with an observed increase of 78% on Day 28 compared to placebo (90% CI +33, +139, probability=0.999) (Fig. 3). In addition, there was a significant increase in serum CXCL8 at Day 14 when compared to baseline (+84%; 90% CI +60, +111) and to placebo (+84%; 90% CI +56, +117), which appeared to be dose-dependent, unlike fibrinogen and CRP where 20 mg did not differ from placebo (Fig. 3). The increase in CXCL8 did not increase further at Day 28.

There were no significant differences in other blood biomarkers, including CC-16, SP-D, MMP-8 and MMP-9.

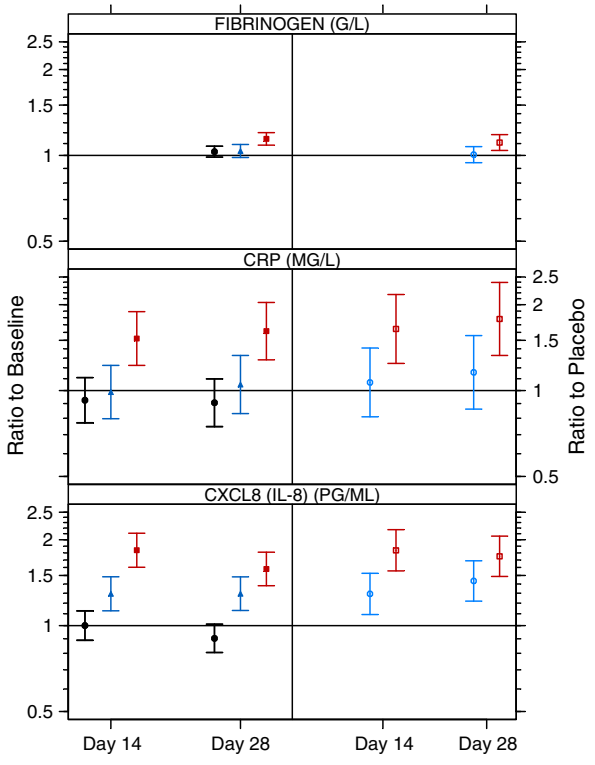

Fig. 3. The effect of SB-656933 on blood biomarkers at Day 14 and Day 28. Left: adjusted geometric mean±90% CI of the ratio to baseline for fibrinogen (top), C-reactive protein (middle) and CXCL8 (bottom). ●—placebo, ♦—SB-656933 20 mg, ■—SB-656933 50 mg. Right: adjusted geometric mean±90% CI for treatment ratio to placebo for SB-656933 20 mg (○) and SB-656933 50 mg (□).

These data are summarized in the online supplement (Table S2). The results of the TOBIT analysis were in line with the results from the analysis using the imputation method. Exploratory analysis of age and gender effects revealed no consistent pattern. For example, females reported higher MPO levels compared to males, whereas age had a significant effect on CXCL8. Further exploration of the data did not show any clear reason for a significant age effect (data not shown).

One-sided multivariate analysis of sputum and blood biomarkers was performed. A first cluster of lung-derived markers included sputum total cell count, sputum NE and sputum MPO (both adjusted for total protein), sputum weight, free DNA, sputum PGP and serum SP-D. A second cluster included neutrophil/CXCR2-associated markers: sputum NE, sputum MPO, sputum neutrophil number, serum IL-8 and serum MMP-8. The lung-derived cluster did not show a significant improvement for SB-656933 20 mg when compared to placebo (p=0.450). However, there was a significant improvement (at the 10% level) for SB-656933 50 mg when compared to placebo (p=0.065) on Day 28. There was no significant improvement for either SB-656933 treatment compared to placebo for the second cluster of neutrophil/CXCR2-associated endpoints on Day 28.

3.4. Lung function

There was a non-significant decrease in FEV_1 for SB-656933 20 mg (−0.062 L, 90% CI −0.150, +0.026) and for SB-656933 50 mg (−0.044 L, 90% CI −0.131, +0.044) when comparing active treatment with placebo at Day 28, although the study was not powered to detect a difference. There was no clinically significant change in FVC for SB-656933 20 mg or 50 mg when compared to placebo (data not shown).

3.5. Pharmacokinetics

A summary of the pharmacokinetic parameters for SB-656933 20 mg and 50 mg is presented in Table 3. Pharmacokinetic results differed markedly from previous clinical investigations of SB-656933 [14], including the pharmacokinetic parameters determined from a separate group of nine CF patients who participated in a previous study (http://www.gsk-clinicalstudyregister.com). PK parameters between each sampling occasion (Days 1 and 28) were similar, indicating that there was no notable accumulation of SB-656933, which would not have been predicted based on prior data with this compound. On Day 1 and Day 28, the average plasma SB-656933 concentrations were less than IC_{50} (331 ng/mL) following SB-656933 20 mg dosing, and only breached IC_{50} for approximately 4 h following SB-565933 50 mg dosing.

3.6. Health outcomes

The most commonly reported symptoms were cough, productive cough and feeling tired (data not shown). Some improvements in daily symptoms were observed in all treatment groups, but categorical analyses suggested that the numbers with improvement were too small to be meaningful. Decreases in frequency were

Table 3
Derived plasma pharmacokinetic parameters of SB-656933.

Parameter	Treatment	Visit	N	Geometric mean (SD logs) [a]	95% CI of geometric mean	% CVb
AUC_{0-4} (ng.h/mL)	SB-656933 20 mg	Day 1	44	741.1 (0.585)	625.4–892.3	63.8
		Day 28	38	592.3 (0.790)	456.8–768.0	93.1
	SB-656933 50 mg	Day 1	41	2227.6 (0.428)	1946.2–2549.7	44.8
		Day 28	39	1906.8 (0.738)	1501.2–2421.9	85.1
AUC_{0-t} (ng.h/mL)	SB-656933 20 mg	Day 1	44	1272.5 (0.408)	1124.0–1440.5	42.6
		Day 28	38	592.3 (0790)	456.8–768.0	93.1
	SB-656933 50 mg	Day 1	41	3537.6 (0.430)	3089.1–4051.3	45.0
		Day 28	39	1906.8 (0.738)	1501.2–2421.9	85.1
C_{max} (ng/mL)	SB-656933 20 mg	Day 1	44	342.89 (0.502)	294.31–399.48	53.6
		Day 28	38	222.86 (0.957)	162.71–305.24	122.4
	SB-656933 50 mg	Day 1	41	967.78 (0.509)	824.22–1136.35	54.3
		Day 28	39	778.04 (0.595)	641.57–943.53	65.2
T_{max} (h) [b]	SB-656933 20 mg	Day 1	44	1.94 (0.50–4.00)	–	–
		Day 28	38	2.11 (0.97–4.08)	–	–
	SB-656933 50 mg	Day 1	41	1.74 (0.50–4.25)	–	–
		Day 28	39	1.84 (0.95–4.00)	–	–

[a] Minimum and maximum ranges reported for T_{max}.
[b] Data not log transformed.

observed for some symptoms and some treatment groups, but there was no respiratory symptom for which both active treatment groups demonstrated a sustained decrease from baseline in frequency rank.

4. Discussion

This study was conducted to assess the safety and tolerability of 28 days daily oral dosing with SB-656933 in subjects with cystic fibrosis. When assessing safety, specific attention was paid to the following areas: incidence of CF exacerbations, changes in sputum microbiology, evidence for an effect on peripheral blood neutrophils (neutropenia in particular) and evidence for epididymitis, based on the known pre-clinical profile. The inclusion of a placebo arm was intended to allow for a valid evaluation of adverse events attributable to SB-656933 *versus* those independent of SB-656933. The selected doses had previously been shown to reduce *ex vivo* CXCL1-induced CD11b surface expression, an activation biomarker, on peripheral blood neutrophils from healthy subjects and a small number of adults with CF, and were expected to have pharmacological activity based upon the results of an ozone challenge study in healthy adult subjects [14].

This study demonstrated that SB-656933 is generally well tolerated when doses of 20 mg or 50 mg are administered once daily for 28 days to patients with CF. The drug-related adverse events which led to the premature discontinuation of five subjects resolved prior to study completion, and importantly, the number of subjects who reported a CF exacerbation was comparable across treatment groups.

Laboratory investigations demonstrated that peripheral blood neutrophil counts remained stable throughout the 28 day dosing period, with no instances of neutropenia, consistent with data obtained on repeat dosing in healthy volunteers. Overall, the distribution and semi-quantitative density of *P. aeruginosa* and *S. aureus* in sputum did not change following 28 days dosing with SB-656933. These data need to be interpreted cautiously, as quantitative bacterial counts were not performed, nor was there screening for the emergence of new bacterial organisms other than *S. aureus* or *P. aeruginosa*.

The increased blood levels of fibrinogen and CRP are not easily explained with the available data. While CRP levels are known to fluctuate in patients with CF, the sustained increase was not associated with specific signs or symptoms of adverse effects in these patients. The increase in CRP was seen at 14 days, and was sustained over the remaining dosing period. In other patient groups, such as chronic obstructive pulmonary disease (COPD) or cardiovascular disease, elevated fibrinogen has been associated with adverse outcomes [15–17]. Whether the same is true in patients with CF is unknown. Further studies are needed to determine how early the increases in CRP and fibrinogen occur and whether they are associated with an increase in IL-6, which was not measured in this study.

For subjects receiving SB-656933 50 mg, there was an approximate 30% decrease in sputum neutrophils compared to baseline levels. The observation that sputum neutrophils also declined in patients on placebo likely contributed to the lack of significance, although the study was not powered to detect a difference. In addition, the unexpected increases in sputum neutrophils in patients on SB-656933 20 mg cannot be explained by the AE profile, and were not associated with higher levels of CRP compared to the other treatment groups. Despite the modest effect on sputum neutrophils, a more consistent decrease in sputum neutrophil elastase and myeloperoxidase was seen in patients receiving SB-656933 50 mg. In addition, while sputum free DNA increased in patients on placebo or SB-656933 20 mg, free DNA levels remained stable or decreased in patients on 50 mg. This could mean that more DNA was released in the absence of inhaled antibiotics; alternatively, this could merely reflect the increased use of Pulmozyme in the 50 mg group. Taken together, these data suggest that SB-656933 50 mg reduced neutrophil activation in the lung, which is consistent with reports that changes in neutrophil-associated biomarkers occur before changes in neutrophil burden in CF sputum [18]. In contrast, sputum PGP levels

were increased in all treatment groups. As PGP is a collagen breakdown product, these data likely reflect ongoing inflammation and tissue remodeling in the lung. We speculate that a decrease in sputum PGP may only be observed after prolonged dosing with more sustained reductions in neutrophil activation.

No significant changes in serum or plasma biomarkers were observed in subjects receiving SB-656933, with the exception of CC-16 and CXCL8. CC-16, a marker of acute lung epithelial cell injury and permeability, was decreased at Day 14 in all subjects, including those on placebo, compared to baseline, but then increased back near baseline by Day 28. Interestingly, the baseline level of CC-16 for all subjects was significantly lower than the reported normal range in healthy volunteers [19]. This is consistent with data obtained in other lung diseases such as COPD [20], as well as in a non-interventional biomarker study performed in patients with CF [21]. In contrast, blood levels of CXCL8 were increased in a dose-dependent manner in subjects receiving SB-656933 (sputum CXCL8 levels were not measured). The etiology of this systemic increase is unclear and does not seem to be associated with any specific adverse event profile. One possibility is that antagonism of the natural CXCL8 receptor may result in demargination of the existing chemokine pool. Alternatively, it is possible that CXCR2 normally scavenges free CXCL8, as has been observed with other chemokine receptors [22] and when the receptor is blocked, this removal mechanism is impaired. There are no published data establishing that blockade of the CXCL8 receptor results in a feedback loop that stimulates agonist production, although exploratory analysis of the current data suggested a relationship between SB-656933 concentration in the plasma and CXCL8 increase (data not shown). In addition, elevated expression of KC, the murine homologue of CXCL8, in bronchoalveolar lavage fluid was observed in mice exposed to cigarette smoke and treated with a small molecule inhibitor of CXCR1/2; this effect was not seen in air-exposed animals treated with inhibitor alone, suggesting the possibility of a feedback loop in the setting of inflammation. However, the increased expression of KC did not affect the ability of the compound to reduce smoke-induced neutrophilic inflammation [23].

The doses selected for this study were expected to inhibit CD11b up-regulation by approximately 50%, based on previous *in vitro* and *ex vivo* studies. Notably, on Day 1 and Day 28, the average plasma SB-656933 concentrations were significantly lower than the exposures previously observed to reduce agonist-induced CD11b expression on neutrophils by 50% [24]. These data are surprising given previous pharmacokinetic data reported during clinical investigations in healthy volunteers [14] and in a single dose study in 9 adult patients with CF (http://www.gsk-clinicalstudyregister.com), where the accumulation ratio was predicted to be 1.6 to 1.8 (healthy subjects) or 1.05 to 2.15 (CF), respectively. Based on these data, dosing for this study was selected on a conservative estimate predicting a 2 fold accumulation, in order to maintain adequate safety margins. The higher than expected clearance of SB-656933, with no evidence of accumulation, may therefore provide some explanation for lack of more robust effect on pharmacodynamic biomarkers and health outcomes for this study. It has been demonstrated previously, for a range of small molecules, that PK parameters in CF patients vary considerably compared to those in healthy subjects. An enabling study in CF patients with this compound ultimately proved inconclusive in this regard, and the observed PK from this study could not have been predicted based upon either the healthy volunteer or limited prior CF patient data. The reason for the difference in pharmacokinetics between the two earlier and present CF subject groups cannot be readily explained, although the clearance and distribution characteristics of the compound were markedly different in the CF population. Future studies with SB-656933 in CF would need to consider dosing regimens of twice or three times daily in order to maximize time at or near the IC_{50}.

In conclusion, SB-656933 was generally well tolerated following 28 days dosing with 20 mg and 50 mg. There was no specific adverse event profile associated with active treatment including exacerbations, changes in sputum microbiology, or peripheral blood neutropenia, although serum drug concentrations were lower than expected. Extensive biomarker sampling suggested reduced sputum neutrophil activation in patients receiving the higher 50 mg dose of SB-656933, although patients receiving the 20 mg dose demonstrated opposite effects, which could not be explained by differences in demographics or pharmacokinetics. While some of the sputum biomarker data are encouraging, longer term dosing at pharmacologically active exposures will be necessary to fully assess the anti-inflammatory potential as well as the significance of elevated systemic inflammation, and to determine whether treatment with SB-656933 affects clinical outcomes such as lung function or respiratory symptoms in patients with CF.

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Conflict of interest statement

ALL, SJM and RTS are employees of GSK and hold stocks in the company. RBM, MWK and JMP have received consulting honoraria and travel expenses from GSK. EK has no declared conflict.

Role of the funding source

The sponsor was responsible for the study design, as well as the collection and analysis of the data. All authors contributed to data interpretation. AL wrote the initial draft of the manuscript; all authors participated in review and revision of the manuscript.

Appendix A. Supplementary data

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